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Project Summary

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Density Levels of Pathogenic Organisms in Municipal Wastewater Sludge—A Literature Review

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This report discusses a critical review of the literature from 1940 to 1980 of laboratory and full-scale studies on density levels of indicator and pathogenic organisms in municipal wastewater sludges and septage. The effectiveness of conventional municipal sludge stabilization processes (mesophilic anaerobic and aerobic digestion, composting and lime stabilization) and dewatering processes (drying beds, lagooning/storage, and sludge conditioning/mechanical dewatering) was evaluated for reducing density levels of indicator and pathogenic organisms. An annotated bibliography presents all citations reviewed, with pertinent abstracts and methods used by researchers.

This Project Summary was developed by EPA's Municipal Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Sludges originating from municipal wastewater treatment plants harbor a multitude of microorganisms, many of which present a potential health hazard. Risk of public exposure to these organisms is possible when sludges are applied to land as a means of disposal. In recognition of this problem, and as required by Section 405 of the Clean Water Act of 1977 (PL 95-217), criteria for the control of infectious disease in the land application of sewage sludge and septic tank pumpings were issued by the U.S. Environmental Protection Agency (EPA) in 40 CFR Part 257 (Federal Register Vol. 44, No. 179, September 13, 1979).

The "Part 257 criteria" specify what minimum treatment of municipal wastewater treatment plant sludges is required prior to land application of the residue. Acceptable treatment methods, termed "Processes to Significantly Reduce Pathogens," are as follows:

- *Aerobic digestion*—Agitation of sludge in aerobic conditions at residence times ranging from 60 days at 15 °C to 40 days at 20 °C, with a volatile solids reduction of at least 38%.
- *Air drying*—Draining and/or drying of liquid sludge on underdrained sand beds, or on paved or unpaved basins in which the sludge is at a depth of 9 inches (22.9 cm). A minimum of three months is needed, two months of which temperature average on a daily basis is above 0 °C.
- *Anaerobic digestion*—Maintenance of sludge in the absence of air at residence times ranging from 60 days at 20°C to 15 days at 35°C to

55°C, with a volatile solids reduction of at least 38%.

- *Composting*—Using the within-vessel, static aerated pile or windrow composting methods, the sludge is maintained at minimum operating conditions of 40°C for five days. For four hours during this period, the temperature exceeds 55°C.
- *Lime stabilization*—Application of lime to sludge in quantities sufficient to produce a pH of 12 after two hours of contact.
- Techniques demonstrated to be the equivalent of the above on the basis of pathogen removals and volatile solids reduction.

An additional category of treatment processes, termed "Processes to Further Reduce Pathogens", was designated in Appendix II of 40 CFR Part 257 as required if (1) affected land is to be used within 18 months of sludge application for the cultivation of food crops and (2) the edible portion of the crop is likely to be exposed to the sludge. These additional processes are:

- High temperature composting
- Heat drying
- Heat treatment
- Thermophilic aerobic digestion
- Irradiation

In 1980, Camp, Dresser and McKee, Inc. (CDM) undertook a literature review of available domestic and foreign data, from 1940 to 1980, of bacteria, viruses and parasites densities in raw municipal wastewater sludges, on the effectiveness of the "Processes to Significantly Reduce Pathogens" and of conventional sludge dewatering techniques (mechanical dewatering/sludge conditioning and sludge storage/lagooning) to reduce levels of these organisms.

The following organisms, categorized into four groupings, were emphasized:

- Indicators—Total coliform, fecal coliform, and fecal streptococcus bacteria; *Clostridum perfringens* (welchii); bacteriophage
- Pathogenic bacteria—Salmonellae, Shigellae, *Pseudomonas* sp., *Mycobacterium* spp., *Candida albicans, Aspergillus fumigatus*
- Enteric viruses—Enterovirus and its subgroups (polioviruses, echoviruses and coxsackieviruses), reovirus and adenovirus
- Parasites—*Entamoeba histolytica, Ascaris lumbricoides, Taenia* spp., *Schistosoma* spp., and others

In addition to reporting density levels in raw sludge and septage, and the effectiveness of conventional sludge treatment processes in reducing density levels, this review also identified design and operating variables that affect process efficiency, compared results of laboratory pilot-scale studies to those of full-scale plants, and contrasted survival of indicator organisms to that of pathogens. Methods used by each researcher to enumerate organisms were also described, and brief summaries were provided of related citations that were encountered but were not actually used in this report.

Density Levels in Raw Sludge

Levels of bacteria, viruses and parasites in raw sludge are presented in Table 1. Note that the densities of pathogenic organisms are several logs less than indicator organisms. Also, there is a noticeable lack of information on the densities of select pathogenic organisms in raw sludges and septages (i.e., lack of parasite organisms data in septages).

Anaerobic Digestion

This process involves biological degradation of complex organic substances present in wastewater sludges in the absence of free oxygen. Primary or secondary sludge, or a mixture of both, is fed continuously or intermittently into an airtight vessel and retained for varying periods of time.

Retention times can vary from 30 to 60 days in low-rate (unmixed) reactors and from 10 to 20 days in high-rate reactors which are mixed and heated to either mesophilic—30 to 38 °C—or thermophilic—50 to 60 °C—temperatures. The digester's performance is indicated by the percent of volatile solids (VS) destroyed. Reduction of VS usually ranges between 35% and 60%, depending on the character of the sludge, detention time and temperature.

Only limited information was found on levels and reductions of densities of organisms in low-rate digesters. Longer detention times and higher temperatures are correlated with greater density reductions. In high-rate digesters at full-scale plants, reductions of greater than 1 log occur in densities of bacteria and viruses, with the exception of *Pseudomonas aeruginosa* (Table 2). Ova and cysts of parasitic tapeworms, flatworms and roundworms (with the exception of *Trichinella spiralis*) were able to survive this digestion process, while parasitic protozoans were reduced to non-detectable levels.

Comparison of laboratory/pilot-scale data to those of full-scale plants generally indicated that greater density reductions are accomplished in the smaller-scale studies. The larger density reductions are attributed to (1) the ability to achieve optimum digestion conditions on a smaller scale, (2) the absence of short circuiting—when fresh sludge (and, with it, high levels of organisms) is allowed to exit—in laboratory/pilot-scale studies, (3) the differences in sensitivity to the effects of anaerobic digestion of laboratory-

Table 1. *Density Levels of Organisms in Raw Sludge and Septage (Average Geometric Mean of Organisms Per Gram Dry Weight)*

Organism	*Primary*	*Secondary*	*Mixed*	*Septage*
Total coliform bacteria	1.2×10^8	7.1×10^8	1.1×10^8	1.4×10^8
Fecal coliform bacteria	2.0×10^7	8.3×10^6	1.9×10^6	1.2×10^6
Fecal streptococci	8.9×10^5	1.7×10^6	3.7×10^6	6.6×10^5
Bacteriophage	1.3×10^5	NR[a]	NR	NR
Salmonella *sp.*	4.1×10^2	8.8×10^2	2.9×10^2	5.1×10^1
Shigella *sp.*	NR	NR	ND[b]	NR
Pseudomas aeruginosa	2.8×10^3	1.1×10^4	3.3×10^3	2.6×10^1
Parasite ova/cysts (total)	2.1×10^2	NR	$<5.0 \times 10^1$	NR
Ascaris *sp.*	7.2×10^2	1.4×10^3	2.9×10^2	NR
Trichiuris trichiura	1.0×10^1	$<1.0 \times 10^1$	0	NR
Trichiuris vulpis	1.1×10^2	$<1.0 \times 10^1$	1.4×10^2	NR
Toxocara *sp.*	2.4×10^2	2.8×10^2	1.3×10^3	NR
Hymenolepsis diminuta	$6. \times 10^0$	2.0×10^1	0	NR
Enteric viruses[c]	3.9×10^2	3.2×10^2	3.6×10^2[d]	NR

[a] *NR = No data available*
[b] *ND = None detected*
[c] *Plaque forming units per gram dry weight (PFU/gdw)*
[d] *$TCID_{50}$ = 50 percent tissue culture infectious dose*

Table 2. *Density Levels of Indicator Bacteria, Pathogenic Bacteria and Enterovirus Following High Rate Anaerobic Digestion at 35°C for 14-15 to 21 Days*

Organism	Density Level[a] per 100 ml	Log Reduction	
		Mean[b]	Range
Total coliform	3×10^{7c}	2.05	1.78 - 2.30
Fecal coliform	2×10^{7c}	1.84	1.44 - 2.33
Fecal streptococcus	9×10^{6c}	1.48	1.10 - 1.94
Salmonella *sp*	3.7×10^{3d}	1.63	0.91 - 2.08
Ps. aeruginosa	6×10^{5d}	0.58	0.15 - 1.0
Enterovirus	7.9×10^{1e}	1.21	1.05 - 1.36

[a] *Arithmetic average of mean (geometric) values*
[b] *Arithmetic average*
[c] *Count per 100 ml*
[d] *Most Probable Number per 100 ml*
[e] *Plaque Forming Units per 100 ml*

grown seeded organisms used in many smaller-scale studies to that of indigenous organisms.

The usefulness of total coliforms, fecal coliforms, fecal streptococci in indicating both densities and reduction of pathogenic bacteria (*Salmonella* sp.) and enteroviruses was evaluated. No correlation was seen between density levels of indicators vs. *Salmonella* sp. or enteroviruses. Some correlation was seen, however, when density reductions of these organisms were compared. Indicator bacteria and *Salmonella* sp. levels were reduced by similar magnitudes, and fecal streptococci appeared to be the most conservative indicator of enterovirus inactivation.

Mesophilic Aerobic Digestion

In this process, wastewater sludge is aerated in tanks at temperatures ranging from ambient to 37°C, commonly for detention times of 10 to 20 days.

Very little research has been conducted on the effects of aerobic digestion on indicator and pathogenic bacteria, enteroviruses and parasites. Bacteria, enteroviruses and parasites all show variable response to the digester environment, such that there is no certainty of even a 1-log reduction in density level.

Mesophilic Composting

This process involves mixing dewatered sludge cake with a bulking agent, such as wood chips, dry compost or shredded municipal refuse, and then shaping the mass into piles, beds or windrows. Due to the activity of the naturally occurring microorganisms, the compost mass will increase in temperature (up to temperatures of between 45 and 65°C) until available food sources are exhausted. The mass then cools, and it is allowed to mature or "cure," in stockpiles.

There are three principal composting systems presently utilized:

- *Windrow*—The compost mass is shaped into long piles, 90 to 150 centimeters (cm) in height, which are turned periodically. This composting process is usually completed in 6 to 10 weeks.
- *Forced aeration* (Beltsville system)—Sludge and woodchips are formed into piles about 360 cm high for a period of 21 to 28 days. During this time, air is blown or pulled through the pile.
- *Closed system*—The compost mass is mixed and aerated in a rotating drum, or in moving elevators, for two to three weeks. During this time, temperatures commonly reach 70°C.

A fourth composting technique, the deep-pile bin system, has been used experimentally. The technique utilizes aerated bins measuring 300 cm on each side and 300 cm in height, with the compost mass turned periodically.

In this review of composting data, it was found that most researchers operated systems at significantly higher temperatures and over much longer time periods than are defined by EPA for composting as a process that significantly reduces pathogens (a minimum temperature of 40°C throughout the composting period, with a temperature of 55°C attained for at least four hours).

High temperatures generated by microbial activity in the composting process can inactivate or destroy many microorganisms present in sludge. Within the protocol for mesophilic composting, however, the temperatures attained are not instantly lethal to most indicator and pathogenic microorganisms of concern. The effectiveness of the process depends, therefore, not on temperature alone, but rather on maintaining the moderately high temperatures throughout the composting mass over a set period of time. Whenever elevated temperatures are not uniformly attained through the compost mass, subsequent mixing of the mass can cause bacterial populations from low-temperature zones to reinoculate areas where bacteria had been inactivated by higher temperatures. In open-windrow or forced-aeration systems, maintenance of a uniformly high temperature is difficult. The "toe" or lower outer edge of a static pile used in forced aeration composting typically remains cooler than the inner portion of the pile. In open windrow composting, turning the pile will cause variations in the heating and cooling of the pile. Generally, in the closed composting system uniform temperatures can be routinely maintained.

Information on density reductions of indicator and pathogenic bacteria, viruses and parasites was drawn from both laboratory, pilot and full-scale studies. Total coliform and fecal coliform bacteria density levels decline by more than 3 and 4 logs, respectively. Fecal streptococcus appear to be quite resistant to conditions of mesophilic composting, with regrowth evident.

Salmonella densities are reduced by approximately 1 to 3 logs during mesophilic composting, generally resulting in densities of less than, or equal to, 10 organisms per gram dry weight of sludge (on a Most Probable Number basis, or MPN). *Shigella sonnei*, *Staphylococcus aureus* and *Serratia marcescens* are also significantly reduced in number, but *Mycobacterium tuberculosis* will apparently survive. Mesophilic composting will not significantly reduce densities of the fungus *Aspergillus fumigatus*; in fact, the temperatures encountered are optimum for this organisms' growth.

Most viruses of concern appear to be quite vulnerable to the temperature conditions of composting. Echo, reo and coxsackie virus densities are reduced by three logs by temperatures within the mesophilic range, as are the adenovirudae. Poliovirus appears to be similarly susceptible. A bacteriophage

(f_2) when added to sludge was found to be far more resistant to mesophilic temperatures and, therefore, reductions in levels of this non-pathogenic and easily cultured virus could provide a useful indication of enterovirus inactivation.

Ova of the roundworm, *Ascaris lumbricoides*, can survive at temperatures higher than those specified for mesophilic composting, presenting a potential problem in sludge treated by mesophilic composting.

Lime Stabilization

Lime is mixed with sludge in quantities sufficient to raise the pH to 12.0 for at least two hours. Lime may be added (1) to liquid sludge prior to dewatering, (2) directly to a mixed-sludge storage tank, followed by land application; or (3) to a dewatered sludge cake. The technique most commonly used by the researchers whose data were utilized in this review involved the addition of lime to liquid sludge.

Lime stabilization can effect significant reductions in levels of some indicator and pathogenic bacteria and, possibly, of poliovirus. The effectiveness has been shown to be contingent upon the pH achieved in the stabilization protocol. It appears that different bacteria respond differently to increasing levels of pH achieved in the process. Even after an effective pH level is achieved in sludge, the decrease in pH level that occurs after the initial exposure and minimum contact time can create an environment favorable to regrowth of some bacteria.

Fecal coliform, *Salmonella* spp. and *Pseudomonas aeruginosa* density levels all appear to be reduced by 2 logs or greater at pH 11 or above. There is no apparent tendency for these microorganisms to regrow. Fecal streptococcus, however, are more resistant to lime inactivation and are able to regrow quickly with decreasing pH to near original densities or greater within 24 hours.

In one study, lime treatment of sludges inactivated *Ascaris* eggs, however, the lime concentrations and time needed were substantially greater than is normally used for sludge conditioning. Also, this inactivation of *Ascaris* eggs was not always consistent and therefore cannot be relied upon.

Conventional Sludge Dewatering Processes

Drying Beds

In general practice, digested sludge is placed on sand beds or paved beds that have been provided with drainage. The sludge is allowed to dry to approximately 40% solids content, over a period of about 10 to 15 days (under favorable conditions) — a significantly shorter time period than the minimum of three months delineated by the EPA.

Information was found on the inactivation of bacteria, viruses and parasites during drying, but none of the data conformed to the criteria specified by the EPA. The research conducted does, however, focus attention on the solids level achieved during drying. This parameter could be useful, in addition to time, temperature, and sludge depth, as an additional criterion for defining air drying of sludge.

Sludge Storage/Lagooning

In this process, anaerobically or aerobically digested sludge is stored in earth- or concrete-lined lagoons at depths of from 60 to 600 cm for periods ranging from several months to years. The performance of the lagoons is affected by climate; both precipitation and low temperatures will inhibit dewatering and the rate of volatile solids reduction. The two factors that have been studied with regard to survival of indicators, pathogens and parasites are temperature and length of storage time. At lower temperatures, a longer detention time is required to achieve reduction of density levels.

It was concluded, based on trends indicated by the data reviewed, that at temperatures of 20°C or greater, the minimum storage time required to achieve a 1-log density reduction is one month for bacteria, two months for viruses, and greater than six months for parasitic ova. At temperatures of less than 20°C, more than six months' storage is required to reduce density levels of pathogenic bacteria by 1 log, more than eight months for viruses, and at least three years for parasitic ova.

Sludge Conditioning/ Mechanical Dewatering

For purposes of this review (and as commonly practiced), dewatering involves use of vacuum filter, chamber filter press, belt filter press, or centrifuge to separate the liquid and solid components of sludge. Typically sludge cake solids content of 15 to 40% are achieved. Chemical conditioners used to aid sludge dewatering include lime (CaO), ferric chloride ($FeCl_3$), ferrous sulfate ($FeSO_4$), and polyelectrolytes or polymers.

The process of mechanical dewatering of municipal wastewater sludges alone has little effect on the density levels of pathogens. The conditioners that are commonly used in combination with mechanical dewatering vary in effects. Polymer has no apparent effect on density levels of pathogens. Lime, added in concentrations to optimize dewatering, cannot be relied on to reduce pathogen levels because of the variations in pH levels obtained. Ferric chloride, often used in conjunction with lime, appears to reduce whatever virucidal and bactericidal effects the lime normally has when applied to sludge.

Conclusions and Recommendations

Because a large body of literature containing comparable data is not available, it is recommended that additional research be conducted on the effectiveness of sludge treatment processes in reducing density levels of organisms. It is recommended, further, that researchers document carefully all pertinent aspects of their experimental design.

The following conclusions appear to be valid based on the literature reviewed:

- Anaerobic digestion and lime stabilization consistently produce reductions of about 1 to 2 logs in densities of indicator and pathogenic bacteria and, in the case of anaerobic digestion, in densities of viruses as well. At a minimum, effectiveness depends on the processes being carried out under the conditions specified in 40 CFR Part 257. Neither sludge stabilization process appears to be particularly effective for inactivating parasite organisms.
- Conditions of mesophilic composting may inactivate common indicator and pathogenic bacteria and viruses, provided that specified temperatures are attained uniformly throughout the compost mass for over the specified time period. The pathogenic fungus *Aspergillus fumigatus* thrives under conditions

of mesophilic composting, however, and parasite ova appear to survive this process.

- Density reductions of bacteria by aerobic digestion are variable and of relatively small magnitude. However, there is a lack of data on the performance of this process and also of air drying in reducing densities of microorganisms.
- Sludge lagoons can achieve 1-log reductions in densities of bacteria and viable parasite ova, but, depending on conditions, storage of one month to more than three years may be required.
- Mechanical dewatering of sludge, with or without the use of chemical conditioners, has little reliable effect on densities of pathogens.

Few of the laboratory-scale studies reviewed could be related to results obtained at full-scale treatment plants. Operating parameters used in laboratory experiments differed radically from those at full-scale plants. For this reason, comparing density levels was seldom possible. In addition, laboratory studies often used seeded bacteria, viruses, or parasites and it is doubtful whether their behavior mimics that of naturally occurring organisms.

No single indicator organism (either bacteria or bacteriophage) was found to maintain a density level of a constant relative value to that of pathogenic organisms. The data available made it impossible to determine whether this inconsistency is due to the inability of current techniques to enumerate pathogenic bacteria and enteroviruses accurately, or to the fact that densities actually vary.

Of the traditional indicators, fecal streptococci appear to be the most conservative indicator of both the density levels of pathogenic bacteria and enterovirus in raw sludge and of their inactivation during sludge treatments. Additional research is required to identify other indicator systems, both bacterial and viral, whose numbers better reflect both density and reduction of density levels of pathogenic organisms.

A wide variety of methods were used to enumerate all of the organisms considered in this review. Although standard methods are available for quantifying the coliform and streptococcus bacteria and for *Salmonella* sp., there are no standard techniques for other pathogens, enteroviruses, or parasites. It is recommended that this area be addressed so that comparable data can be produced in future studies.

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The complete report, entitled "Density Levels of Pathogenic Organisms in Municipal Wastewater Sludge—A Literature Review," (Order No. PB 82-102 286, Cost: $21.50, subject to change) will be available only from:
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